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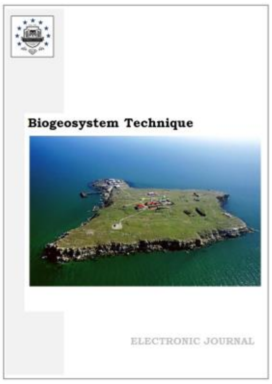

Articles

Diagnostics of the Content of Petroleum Products and Heavy Metals in Light Chestnut Soils

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Abstract

The article presents the diagnosis of contamination of light chestnut soils of Volzhsky, Volgograd region with petroleum products and heavy metals using various bioindicators: earthworms of the genus *Lumbricus rubbellus* and fungus of the genus *Botryitis cinerea*. The following indicators of bioindication of soil pollution were identified: the survival rate of the earthworms *Lumbricus rubbellus*, the total microbial number of light chestnut and the mycelium mass of the fungus of the genus *Botrytis cinerea*. In clay and sandy light chestnut soils that are contaminated with oil and petroleum products, the mortality of earthworms of the genus *Lumbricus rubellus* was noted on day 7. After 7 days, the spores of the *Botrytis cinerea* fungus began to grow on the control and light chestnut sandy soil of gas station number 3, and after 14 days – on other soils. The survival rate of earthworms depends on the quality of petroleum products and does not depend on the granulometric composition of soils. The largest total microbial number of light chestnut soil, estimated by the Botrytis cinerea fungus, is observed in the sandy soil of gas station number 3 (133333 CFU/cm³ of water soil extract), and the smallest – in the clay soil of gas station number 1 (6773 CFU/cm³ of water soil extract).

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Keywords: light chestnut clay and sandy soil, bioindication, petroleum products, heavy metals, earthworms of the genus *Lumbricus rubbellus* and fungus of the genus *Botrytis cinerea*.

1. Introduction

The change in the physical properties of soils under the influence of oil and petroleum products pollution is characterized by the aggregation of soil particles, the water properties and air regime of soils deteriorate (Hewelke et al., 2018, Adams et al., 2008, Błońska et al., 2016, DeBano, 2000, Dekker, Jungerius, 1990, Gordon et al., 2018, Marín-Garcíaetal., 2016, VanDeSteene, Verplancke, 2007, Ferguson et al., 2003, Eckert-Tilotta et al., 1993).

Changes in the chemical properties of the soil during contamination with oil and petroleum products are characterized by the following parameters: the content of organic carbon increases; the soil solution is alkalinized (Okolelova, Egorova, 2020, Okolelova et al., 2015, Xi et al., 2021, Robichaud, 2019).

The composition of oil and petroleum products includes naphthenic-aromatic polycyclic, dicyclic and monocyclic hydrocarbons, cycloparaffins, paraffins. Representatives of these classes of compounds are contained in any soil, regardless of its type, in the form of non-specific organic compounds, in humic and fulvic acid molecules. Therefore, it is impossible to establish the maximum permissible concentrations for petroleum products. A similar opinion is shared by other authors (Vorobeychik, 2011, Sukhanosova et al., 2009).

The duration of the adaptation period in soils contaminated with petroleum products varies greatly in different climatic conditions and also depends on the concentration of oil in soils (Wesselink et al., 2009).

Studies by many authors have proved the influence of oil and petroleum products on the growth and development of plants (Gamm, Maslova, 2012, Pashayan et al., 2021, Adesina, Adelasoye, 2014, Ammosova, Golev, 1998, Holt, 1987, Klamerus-Iwan et al., 2015, Langer et al., 2010, Walker et al., 1987).

To date, there are many works devoted to various methods and methods of cleaning soils and waters from oil and petroleum products pollution (Rogozina et al., 2010; Guslavsky, Kanarskaya, 2011, Szarlip et al., 2014, Muratova et al., 2012, Schoefs et al., 2004, Vasilyeva et al., 2021).

Earthworms of the genus *Lumbricus rubbellus* are special representatives of soil–dwelling animals (Figure 1), swallowing soil and passing it through themselves (Prokhorov, 2004).

Fig. 1. Earthworms *Lumbricus rubbellus* in their natural habitat

The most optimal conditions for the active life of earthworms: pH = 5.5-8.7 (they prefer a neutral soil environment); temperature – 10.6 ° C; humidity – 70-85 %). Such a range of humidity is close to the water content in the body of the worm, while their development worsens soil moisture below 30-35%, and soil moisture equal to 22% is fatal for them. The acidity of the medium also affects the vital activity of worms: if it is below pH = 5 or above pH = 9, then all worms die within a week. A salt concentration of more than 0.5 % is fatal for them. The presence of earthworms increases the concentration of microorganisms producing vitamin B in the soil. This contributes to an increase in organic matter (Gimadeev, Shchipovskikh, 2000, Prokhorov, 2004).

Previously, we studied the condition of soils contaminated with oil and petroleum products using earthworms of the genus *Lumbricus rubellus* as a test organism. As a result of these experimental studies, an inverse relationship was established between the quality of fuels and lubricants (automobile gasoline AI-92, AI-95 and locomotive diesel fuel) and the survival rate of worms. The behavioral reaction of earthworms of the genus *Lumbricus rubellus* when polluted with AI-92 and AI-95 gasoline is manifested in the form of twisting worms into a ball, and when polluted with locomotive diesel fuel – in burying worms in the soil (Zaikina et al., 2016, Okolelova, Zaikina, 2017).

We have also conducted research on detoxification of oil-contaminated soils using chitosan.

Chitosan is a natural sorbent, one of the important features of which is the possibility of biodegradation and decomposition under the action of enzymes (Kokorina et al., 2012).

The fungus of the genus *Botrytis cinerea* was chosen as a culture for determining the biodiagnostic indicators for assessing soil contamination with heavy metals and petroleum products due to the fact that it occurs on various plants and can affect the soil, and it also has a good ability to grow in laboratory conditions (Zaikina et al., 2018, The world of plants, 1991, Fernández-Acero et al., 2010, Hoeberichts et al., 2003, Lin Zhang et al., 2008, Staats et al., 2005).

Botrytis cinerea is a phytopathogenic fungus infecting a number of agricultural crops (tomatoes, strawberries, grapes, etc.), which has been adopted as a model system in molecular phytopathology. It is the causative agent of gray rot on plants, which develops in the conidial and sclerotic stages. Gray plaque on the affected berries and other parts of the plant is a manifestation of the conidial stage of sporulation. The sclerotic stage is expressed by the formation of irregular sclerotia, black in color, 2-5 mm in diameter on berries, leaves and other parts of plants under unfavorable conditions (depletion of the nutrient substrate, high or low temperatures, drought) (Hoeberichts et al., 2003, Lin Zhang et. al., 2008, Staats et al., 2005).

The strain of Botrytis cinerea culture was provided by the Laboratory of Mycology and Phytopathology of the All-Russian Research Institute of Plant Protection (FGBNU VISR). Fungi of the genus Botrytis cinerea in laboratory (A-D) and natural conditions (E) are shown in Figure 2.

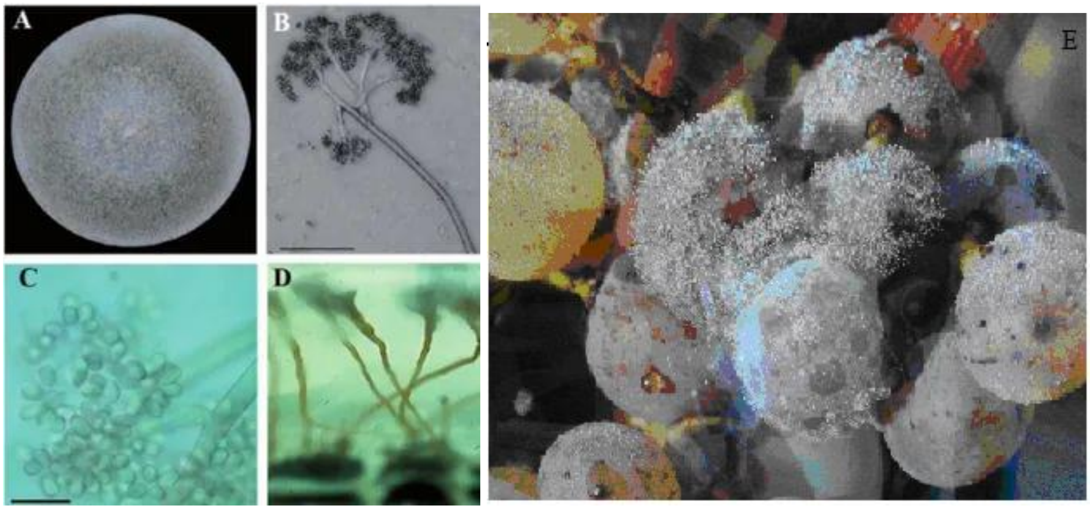

Fig. 2. Fungi of the genus *Botrytis cinerea* in laboratory (A-D) and natural conditions (E)

2. Materials and methods

The objects of the study are located in Volzhsky of Volgograd region. These are light chestnut sandy (gas station No. 3) and clay (gas station No. 1) soils. Sampling and preparation of soils for analysis was carried out according to GOST 17.4.3.01-2017 (GOST 17.4.3.01-2017, 2018).

Determination of pollutants in soils

The gross forms of Cu, Ni, Zn were determined by the X-ray fluorescence method on the device "Spectroscan MAX-GV" according to GOST 33850-2016 (GOST 33850-2016, 2019) they are presented as the sum of HM_{Σ}. The analysis of water–soluble forms of chemical elements was carried out: Cu and Ni – on the device "Spectrophotometer UNICO 2100" by photometric method, Zn - on the liquid analyzer "Fluorat-02-3M" by fluorimetric method. The content of petroleum products (PP) in the soil and water extract was determined on the device "Concentration meter KN-2M". The concentration of organic carbon (humus) was determined by the I. V. Tyurin method in the modification of the TSINAO on the SF-14 spectrophotometer with an error of 0.26-0.55 % according to GOST 26213-91. The data were obtained earlier and are shown in Table 1 and Table 2.

Experimental studies to determine diagnostic indicators of soil pollution using earthworms of the genus *Lumbricus rubellus*

The test organism was the earthworms of the genus Lumbricus rubellus, since this species is sensitive to petroleum products and heavy metals, typical of zonal soils of the Volgograd region, and allows you to quickly determine the presence of petroleum products and heavy metals.

The model experiment with light chestnut sandy soil from the village of Bereslavka in the Gorodishchensky district of Volgograd was carried out three times in plastic cuvettes with a volume of 500 ml, into which 400 g of soil, 30 ml of distilled water, 1 or 10 ml of AI-92 and AI-95 gasoline, locomotive diesel fuel were introduced (2 variants of the experiment) and then 10 earthworms were brought to the surface in each cuvette with contaminated soil and their behavioral reactions and survival (mortality) were monitored (Kozlov, 2003, Fomin G.S., Fomin A.G., 2011).

To assess the effect of petroleum products on the behavior and survival of earthworms, 12 transparent plastic cuvettes with a volume of 500 ml were used for soil from gas stations No. 1, 3. The experiments were carried out twice. 400 g of clay soil from gas station No. 1 was placed in two cuvettes, 400 g of sandy soil from gas station No. 3 was placed in the other two cuvettes, and 30 ml of water was introduced into each cuvette. 400 g of clay soil from gas station No. 1, 30 ml of water, 30 g of chitosan were placed in the next two cuvettes. 400 g of sandy soil from gas station No. 3, 30 ml of water, 30 g of chitosan were placed in the other two cuvettes. The control consisted of two cuvettes, into which 400 g of chestnut clay soil and 30 ml of water were introduced.

10 earthworms (*Lumbricus rubellus*) were introduced to the surface in each cell. The experiments were carried out at a temperature of 19-21 ° C. Survival was determined taking into account the mortality of worms during the experiment.

Experimental studies to determine diagnostic indicators of soil pollution using a fungus of the genus *Botrytis cinerea*

A pre-prepared water extract from 2 soil samples was prepared according to the method of E.V. Arinushkina according to GOST 26423-85 (GOST 26423-85, 2011): 50 g of air-dry soil was quantitatively transferred to a flask, 250 ml of distilled water was added, then the container was vigorously shaken for 3 min., after shaking, the entire suspension of the soil was filtered.

To cultivate *B. cinerea*, laboratory utensils and culture medium were sterilized, respectively, by dry heat in an autoclave at a pressure of 1.0 atm (Tepper et al., 2004). Then 50 ml of culture medium and various dilutions from water extracts of 2 soil samples were added to the flasks in 3-fold repetition: 10^{-2}, 10^{-3}, 10^{-4}. 50 ml of culture medium was introduced into the control flasks under sterile conditions. After that, a suspension of *B. cinerea* conidia was prepared in a culture medium containing 10^3 CFU/ml, and 250 mkl of suspension was added to each flask. Chapek's medium was used as a liquid culture medium. After 10-14 days, the total microbial number (TMN) of the soil was calculated and compared with the control (Tepper et al., 2004).

The calculation of the total microbial number (TMN) of the soil can be expressed by the formula:

$$TMN = \frac{X \times P}{V},$$

where TMN is the total microbial number of the soil, CFU in 1 cm³ of the aqueous extract of the soil; X is the microbial number, CFU in 1 cm³ of the medium for cultivating the fungus *B. cinerea* (a mixture of nutrient medium and aqueous extract of the soil); P is 1/degree of dilution; V is the volume of the medium for cultivating the fungus *B. cinerea*.

3. Results and discussion

Diagnostics of soil contamination with petroleum products and heavy metals using earthworms *Lumbricus rubbellus*

It is known that the duration of the adaptation period in soils contaminated with petroleum products varies greatly in different climatic conditions and also depends on the concentration of oil in soils (Sukhasonova et al., 2009). A model experience using earthworms of the genus *Lumbricus rubbellus* is illustrated in Figure 3.

Fig. 3. Model experience using earthworms of the genus *Lumbricus rubbellus*

Previously, we have shown the effectiveness of using chitosan of various aggregate composition for detoxification of soils contaminated with oil and petroleum products. Its use reduces the content of PP in light chestnut sandy soil by 1.2-4.8 times, in light chestnut clay by 10.2-77.4 times (Kokorina et al., 2012).

A model experiment with the earthworms Lumbricus rubbellus

Observations showed that in cuvette, with the AI-92 gasoline introduced, the death of all earthworms occurred 2 minutes after the start of the experiment. In the first minute, 7 earthworms died in the first cuvette, 5 in the second, 6 in the third. The behavioral reaction in the first minute was a sharp twisting of the earthworm into a ball.

In a plastic cuvette with AI-95 gasoline in all three cuvettes, all worms died after 3 minutes. In all three cuvettes, all 10 worms were alive in the first minute. The behavioral reaction is a sharp twisting of the worm into a ball. After 2 minutes have passed since the beginning of the experiment, 6 worms out of 10 died in the first cuvette, 8 died in the second cuvette, and 7 died in the third cuvette.

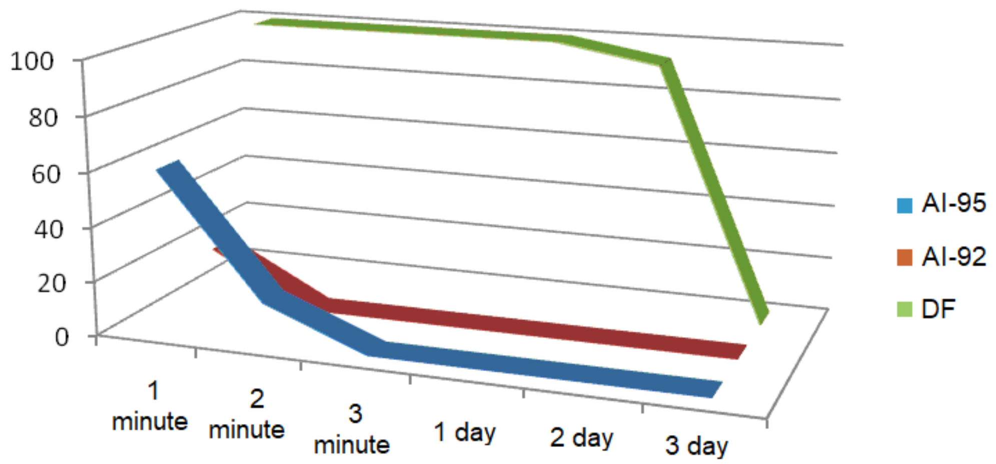

Fig. 4. Graphical dependence of mortality of earthworms *Lumbricus rubellus* with 1 ml of fuel and lubricants

In three cuvettes with diesel fuel (DF) introduced, the death of worms occurred after 3 days. In the first cuvette on the second day, 1 worm out of 10 died. In the second and third cuvettes, all 10 worms were alive. Behavioral reaction – worms were actively crawling in all three diesel fuel cuvettes, there was an effect of burrowing into the soil.

In the reference samples (control), all ten worms actively crawled on the soil surface. The death of worms within 30 days was not detected. The results of the experimental model experience are presented in Figure 4.

Based on the graph shown in Figure 4, it can be seen that the mortality of worms occurs at the 3rd minute in experiments with AI-95, at the 2nd minute – with AI-92 and on the 3rd day when diesel fuel (DF) is introduced.

Our experiments showed that the survival rate of earthworms of the genus *Lumbricus rubellus* was the lowest percentage in the experiment with gasoline AI-92. In the first variant of the experiment (10 ml), the total mortality of worms was recorded 1 minute after its start, the survival rate was 0 %, in the second variant (1 ml) – after 2 minutes. The survival rate in the 1st minute was 40 %, in the 2nd minute – 0.

In second place in the survival rate of worms was an experiment with gasoline AI-95. The total mortality of worms in the first experiment was 2 minutes after the start of the experiments. The survival rate in the 1st minute was 20 %, in the 2nd – 0. In the second experiment, the survival rate in the 1st minute is 100 %, in the 2nd – 30 %, in the 3rd minute – everyone died. The greatest survival rate was recorded in experiments with locomotive diesel fuel (DF). In the first experiment, the total mortality of worms was recorded on day 3. The survival rate on the 1st day was 100 %, on the 2nd day – 90, on the 3rd day – all died. In the second experiment, the complete death of *Lumbricus rubellus* was also observed on the third day. The survival rate on the 1st day was 100 %, on the 2nd day – 96.7 %, on the 3rd day – all died.

In the control experiment, the death of *Lumbricus rubellus* within 30 days was not detected.

Experiment without chitosan

For the first three days, the worms were alive in all the ditches. On the fourth day, 2 out of 10 worms died in the soils taken from gas station No. 1 and gas station No. 3. On the fifth day, 6 out of 10 worms died in the sandy soil from gas station No. 3, in the clay soil from gas station No. 1 – 5 out of 10 worms. On the sixth day, 8 worms died in the soil from gas station No. 1, 9 worms died in the soil from gas station No. 3. A week later, all 10 worms died in experiments without chitosan (Table 1).

Table 1. Survival of earthworms of the genus Lumbricus rubbellus in oil-polluted light chestnut soils, %

Experience time, days	Number of surviving worms								
	Control			Without chitosan			With chitosan		
	I	II	Average	I	II	Average	I	II	Average
1	2	3	4	5	6	7	8	9	10
Clay soil, Gas station No. 1, PP = 135 mg/kg, HM_{Σ} = 188.19 mg/kg									
1	10	10	10	10	10	10	10	10	10
4	10	10	10	8±0,71	9±0,71	8±0,71	10	10	10
5	10	10	10	4±1,41	6±1,41	5±1,41	10	10	10
6	10	10	10	2±0,71	3±0,71	2±0,71	10	10	10
7	10	10	10	0	0	0	10	10	10
31	10	10	10	0	0	0	7±0,71	8±0,71	7±0,71
Sandy soil, Gas station No. 3, PP = 202 mg/kg, HM_{Σ} = 242.74 mg/kg									
1	10	10	10	10	10	10	10	10	10
4	10	10	10	9±0,71	8±0,71	8±0,71	10	10	10
5	10	10	10	5±0,71	4±0,71	4±0,71	10	10	10
6	10	10	10	2±0,71	1±0,71	1±0,71	10	10	10
7	10	10	10	0	0	0	10	10	10
31	10	10	10	0	0	0	6±0,71	7±0,71	6±0,71

Experiment with chitosan

Within a week, all the worms were alive. After 31 days, 3 worms died in the clay soil from gas station No. 1, 4 – in the sandy soil from gas station No. 3. In the control, no dead worms were observed for 31 days (Table 1).

The analysis of the received data shows the following. The content of petroleum products and heavy metals in the soil negatively affects the test organism (*Lumbricus rubellus*). In clay and sandy light chestnut soils, 100% mortality was noted on the seventh day. Chitosan effectively reduces the toxicity of oil-contaminated soils. Without introducing chitosan into the soil from gas station No. 1 and from gas station No. 3, worms died on the seventh day, and when it was introduced into the soil from gas station No. 1, 3, 3-4 worms died after 31 days.

In the clay soil of gas station No. 1, the concentration of petroleum products is 135 mg/kg, the gross content of heavy metals is as follows: Cu – 55.79 mg/kg, Ni – 55.34 mg/kg, Zn – 77.06 mg/kg (HM_{Σ} = 188.19 mg/kg); in the sandy soil of gas station No. 3, the concentration of PP = 202 mg/kg, gross content of heavy metals: Cu – 37.33 mg/kg, Ni – 43.32 mg/kg, Zn – 162.09 mg/kg (HM_{Σ} = 242.74 mg/kg).

Diagnostics of soil contamination with petroleum products and heavy metals using a fungus of the genus *Botrytis cinerea*

Terms of cultivation

After 7 days, the spores of the *Botrytis cinerea* fungus began to grow on the control and soil of gas station No. 3. And after 14 days they grew on the remaining soils.

The total microbial number (TMN) of the soil (Table 2)

At the control (only on the nutrient medium of Chapek), the TMN was 245333 CFU / cm^3 of the water extract of the soil.

The largest total microbial number of soil was detected at the highest dilution of 10^{-4} in the sandy soil of gas station No. 3 (133333 CFU / cm^3 of water soil extract), which is 1.84 times less than in the control and 5.37 times higher than in the clay soil of gas station No. 1 (24823). The dependence of the total microbial number estimated by the fungus of the genus *Botrytis cinerea* on the concentration of petroleum products (PP) in the aqueous extract of the soil is shown in Figure 5, for heavy metals it is similar.

Table 2. Results of diagnostics of soil contamination with petroleum products and heavy metals using a fungus of the genus *Botrytis cinerea*

An object	Concentra-tion of PP in water extraction of soil, mg/kg	The total concentration of HM in the water extract of the soil (HM_{Σ}), mg/kg	Breeding	TMN of the soil, CFU / cm^3 of water extraction of soil	Mycelium mass of *Botrytis cinerea* fungus, g
1	2	3	4	5	6
Control	-	-	-	245333±36961	0,0850±0,0220
Gas station No. 1	17,00	7,99	10^{-2}	6773±3815	0,0573±0,0010
			10^{-3}	2482±567	0,0466±0,0020
			10^{-4}	24823±5675	0,0683±0,0280
Gas station No. 3	52,00	9,52	10^{-2}	14234±1108	0,0572±0,0010
			10^{-3}	3243±882	0,0514±0,0008
			10^{-4}	133333±25137	0,0800±0,0120

The lowest total microbial number of soil in light chestnut soils was observed at an average dilution of 10^{-3} in the clay soil of gas station No. 1 (2482 CFU / cm^3 of water soil extract), which is 98.84 times lower than the control and 1.5 times lower than in the soil of gas station No. 3 (3243). In light chestnut soils, the following dependence was revealed: the total microbial number (TMN) of the soil is higher in the sandy soil of gas station No. 3 (133333 CFU/cm^3 of water extract of the soil) with a high content of PP = 52.00 mg/kg and HM_{Σ} = 9.52 mg/kg (water–soluble forms of Cu –

1.59 mg/kg, Ni – 2.00 mg/kg , Zn – 5.93 mg/kg) and with the highest dilution of 10^{-4} and lower in the clay soil of gas station No. 1 (24823) with the lowest dilution of 10^{-2} and with a lower content of PP = 17.00 mg/kg and HM_{Σ} = 7.99 mg/kg (water–soluble forms of Cu – 2.26 mg/kg, Ni – 2.00 mg/kg , Zn – 3.73 mg/ kg), which may be due to the granulometric composition of the soil and a high content of Corg (2.70%) and HM_{Σ} = 9.52 mg/ kg in the soil of gas station No. 3 compared with gas station No. 1 – Corg (0.82%) and HM_{Σ} =7.99 mg/kg.

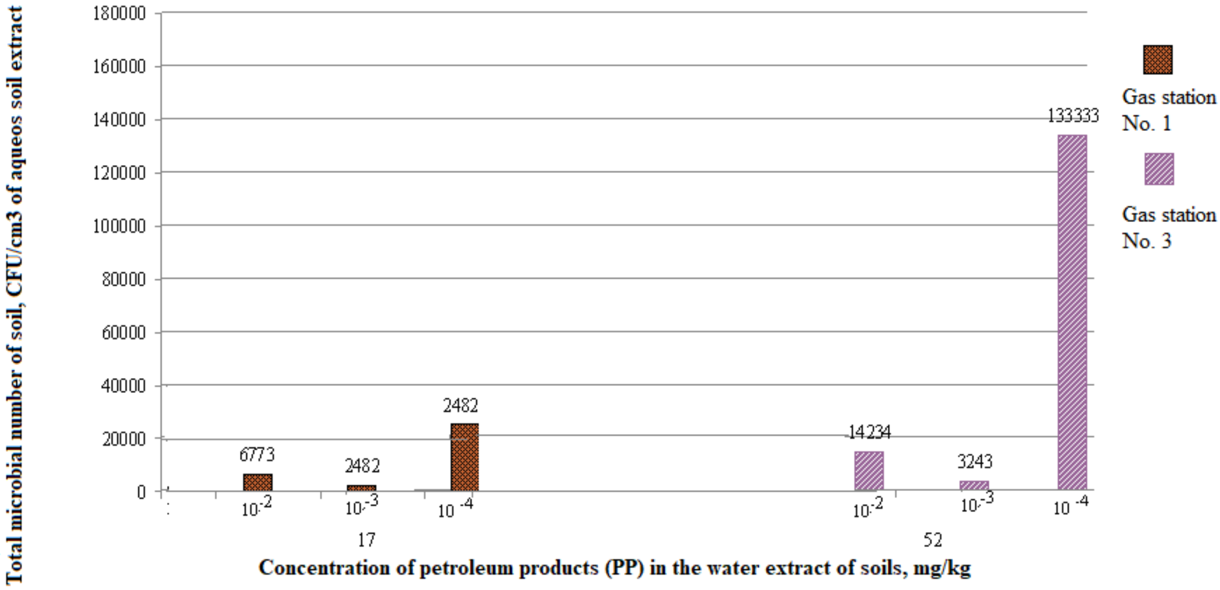

Fig. 5. Dependence of the total microbial number estimated by the fungus of the genus Botrytis cinerea on the concentration of PP in water extraction of soil: 10^{-2}, 10^{-3}, 10^{-4} – dilutions

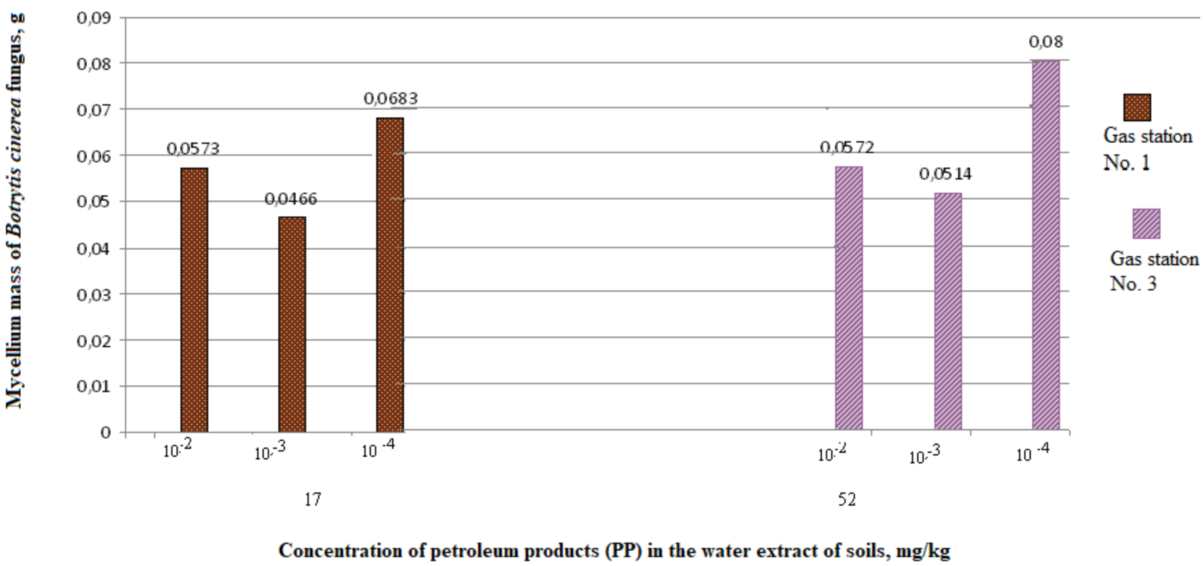

Fig. 6. Dependence of the mycelium mass of the fungus of the genus Botrytis cinerea on the concentration of PP in the aqueous extract of the soil: 10^{-2}, 10^{-3}, 10^{-4} – dilutions

Mycelium mass of the fungus *Botrytis cinerea*

In the control, the mycelium mass of the Botrytis cinerea fungus was 0.0850 g (Table 2). The dependence of the mycelium mass of the *Botrytis cinerea* fungus on the concentration of petroleum products (PP) in the aqueous extract of the soil is shown in Figure 6, for heavy metals it is similar. The maximum mycelium mass of *B. cinerea* fungus was observed at the highest dilution of 10^{-4} in the sandy soil of gas station No. 3 (0.0800 g), which is 0.94 times lower compared to the control and 1.17 times higher than in the clay soil of gas station No. 1 (0.0673). The minimum mycelium mass of *B. cinerea* fungus is observed at an average dilution of 10^{-3} in clay soil of gas station No. 1 (0.0466 g), which is 1.82 times lower compared to the control and 1.10 times lower than in the soil of gas station No. 3 (0.0514).

In light chestnut soils, the following dependence is observed: a large mass of mycelium of the fungus *B. cinerea* was detected in the sandy soil of gas station No. 3 (0.0800 g) with a higher content of PP = 52.00 mg/kg and HM_{Σ} = 9.52 mg/kg (water–soluble forms Cu - 1.59 mg/kg, Ni – 2.00 mg/kg, Zn – 5.93 mg/kg), and its smaller mass is in the clay soil of gas station No. 1 (0.0466 g) with the lowest dilution of 10^{-2} and with a lower content of PP = 17.00 mg/kg and HM_{Σ} = 7.99 mg/kg. This is also possible due to the granulometric composition of the soil and the high content of Corg (2.70%) and HM_{Σ} = 9.52 mg/kg in the soil of gas station No. 3 compared to gas station No. 1 – Corg (0.82 %) and HM_{Σ} =7.99 mg/kg (water–soluble forms Cu - 1.59 mg/kg, Ni – 2.00 mg/kg, Zn - 5.93 mg/kg).

4. Conclusion

The content of petroleum products and heavy metals in the soil negatively affects the survival of the earthworms *Lumbricus rubellus*. In clay and sandy light chestnut soils, 100 % mortality was noted on the seventh day. The survival rate of earthworms does not depend on the content of PP and HM_{Σ} in soils and its granulometric composition. For the first three days, the worms were alive in all the cuvettes. On the fourth day, 2 out of 10 worms died in the soils taken from gas station No. 1 and gas station No. 3. On the fifth day, 6 out of 10 worms died in the sandy soil from gas station No. 3, in the clay soil from gas station No. 1 – 5 out of 10 worms. On the sixth day, 8 worms died in the soil from gas station No. 1, 9 worms died in the soil from gas station No. 3. A week later, all 10 worms died in experiments without chitosan. Chitosan effectively reduces the toxicity of oil-contaminated soils. With the introduction of chitosan into the soil for 10 days in both experiments, the worms lived for 7 days. In the experiment with clay soil of gas station No. 1 (PP = 135 mg/kg, HM_{Σ} = 188.19 mg/kg), 3 worms died on 31 days, 4 worms died on 31 days from gas station No. 3 (PP = 202 mg/kg, HM_{Σ} = 242.74 mg/kg).

After 7 days, the spores of the fungus of the genus *Botrytis cinerea* began to grow on the control and water extraction soil of gas station No. 3. And after 14 days they grew on the water extracts of the remaining soils. The largest total microbial number of light chestnut soil was found in the sandy soil of gas station No. 3 (133333 CFU / cm^3 of water soil extract) with the highest dilution of 10^{-4}, which is 1.84 times less than in the control and 5.37 times higher than in the clay soil of gas station No. 1 (24823). The lowest total microbial number of light chestnut soil was observed at an average dilution of 10^{-3} in the clay soil of gas station No. 1 (2482 CFU / cm^3 of water soil extract), which is 98.84 times lower than the control and 1.5 times lower than in the soil of gas station No. 3 (3243). In light chestnut soils, the maximum mycelium mass of the fungus of the genus *Botrytis cinerea* was noted at the highest dilution of 10^{-4} in the sandy soil of gas station No. 3 (0.0800 g), which is 0.94 times lower compared to the control and 1.17 times higher than in the clay soil of gas station No. 1 (0.0673).

The minimum mycelium mass of the fungus of the genus *Botrytis cinerea* in light chestnut soils is observed at an average dilution of 10^{-3} in the clay soil of gas station No. 1 (0.0466 g), which is 1.82 times lower compared to the control and 1.10 times lower than in the soil of gas station No. 3 (0.0514).

In light chestnut soils, a direct dependence of the total microbial number of the soil and the mycelium mass of the fungus Botrytis cinerea on petroleum products (PP) and heavy metals was found.

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